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Slow Photic and Chemical Induction of Bioluminescence in the Midwater Shrimp, *Sergestes similis* Hansen

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Abstract. The initial luminescent response to photic stimulation of dark-maintained specimens of the midwater shrimp, *Sergestes similis* Hansen, differed from the conventional counterillumination response. Animals were initially unresponsive to light; bioluminescence was only induced after a latency of 3 min. Maximum intensity was reached after approximately 25 min. During the induction process, light emission from the anterior light organs was frequently observed prior to output from the posterior organ. Once luminescence was induced, responses exhibited the typical fast kinetics of the counterillumination response and changes in light organ output occurred synchronously.

Visual input was necessary to maintain this state. Dark readaptation of counterilluminating animals resulted in a return to the slow response kinetics characteristic of untested animals. Because eyestalk ablation or crushing caused immediate production of luminescence in previously untested animals, the slow induction did not involve the ability of the light organs to produce light.

Serotonin was effective in stimulating bioluminescence in intact animals; the induction of light emission proceeded at a rate similar to that for photic stimulation. Other putative neurotransmitters, including norepinephrine, acetylcholine, GABA, and L-glutamic acid, did not stimulate bioluminescence. Isolated light organs exhibited high background levels of light emission, which were unchanged by serotonin treatment. However, serotonin was effective in stimulating luminescence in animals with ablated eyestalks. These results suggest a dual control system involved in the induction and maintenance of bioluminescence in *S. similis*.

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Abbreviations: ACh, acetylcholine; GABA, gamma aminobutyric acid; PCA, p-chloroamphetamine; 5-MT, 5-methoxytryptamine.

Introduction

Marine organisms are vulnerable to predation by upwards-viewing predators that scan for prey silhouetted against downwelling illumination. In some midwater animals, this vulnerability may be reduced by luminescent countershading, or counterillumination, in which downward-directed bioluminescence replaces oceanic light absorbed or reflected by the animal's body (Clarke, 1963; Herring, 1982; Young, 1983). For counterillumination to be optimally effective, light emission must match the spectrum, intensity, and direction of ambient light, so that bioluminescence effectively replaces ambient downward-directed illumination. Strong experimental evidence for a counterillumination role of luminescence exists for midwater squids, fishes, and crustaceans (reviewed by Young, 1983).

Bioluminescence by the decapod shrimp, *Sergestes similis* Hansen, functions in this manner (Warner *et al.*, 1979), counterilluminating the body by matching the spectral distribution (Herring, 1983; Widder *et al.*, 1983), intensity (Warner *et al.*, 1979), and angular distribution (Latz and Case, 1982) of oceanic downwelling illumination. Light emission by *S. similis* associated with counterillumination is stimulated only by downward-directed illumination and can be maintained for long periods (Warner *et al.*, 1979). In the dark, no luminescence is produced for counterillumination.

Little is known of the physiological control of counterillumination. Luminescence by *S. similis* is regulated by visual input; when the eyes are masked, light emission

is absent. Luminescent response latencies to visual stimulation of only a few seconds are consonant with either neural or hormonal control (Warner *et al.*, 1979). Bioluminescence originates from modified portions of the hepatopancreas, the organs of Pesta (Dennell, 1940; Herring, 1981). The mechanism of control of light emission by the organs of Pesta is unclear because the light organs have neither been shown to be innervated nor luminescence to be electrically or chemically excitable (Herring, 1976, 1981).

In other midwater animals, bioluminescence used for counterillumination appears to be under neural control. In squids, morphological and physiological evidence supports direct neural control (Arnold and Young, 1974; Dilly and Herring, 1974; Herring, 1977). The photophores and caudal organs of myctophid fishes are under neural control, even though the chemical basis remains obscure. They are richly innervated and electrically or neurally excitable (reviewed by Herring, 1982).

The present study documents a previously undescribed aspect of counterillumination by *S. similis:* the slow initial induction of luminescence in previously untested, dark-maintained animals, which occurs prior to the counterillumination response. This induction can be mimicked by chemical treatment with the neurotransmitter serotonin. The slow kinetics of photic and chemical induction compared to the typical counterillumination response suggest different mechanisms controlling these responses. Results support the hypothesis that a blood-born factor, perhaps via a neurosecretory pathway, is involved in the induction process.

Materials and Methods

Adult specimens of *Sergestes similis* were collected at night from depths of 75–200 m in the Santa Barbara Basin, near Santa Barbara, California, using a midwater trawl. Trawl contents were recovered under dark conditions on moonless nights and sorted under dim red light. Animals were placed in chilled seawater, brought into the laboratory within 3 h of collection, and were maintained in 100-l aquaria with flow-through, sand-filtered seawater (10°C). All tests were performed within one week of collection, during which time animals remained in good physiological condition and exhibited low mortality. Only actively swimming specimens were used for testing. Except for brief exposure to dim red light during handling, animals remained in constant darkness and were not fed.

For testing, specimens were loosely restrained by a clamp around the cephalothorax and placed in a sealed, clear acrylic chamber (1.75 × 2.5 × 10 cm) filled with 10°C seawater (Fig. 1A). Bioluminescence was induced by downward-directed illumination conducted by a fiber optic light guide from a tungsten-halogen source (Dolan-Jenner Inc.) to a 465 nm interference filter (Ditric Optics, half band width 9.4 nm) and diffused by two opal ground glass plates. Light intensity was regulated by neutral density filters (Rolyn Optics) and measured by a United Detector Technology Inc. 40× Optometer. Stimulus duration was controlled by an electro-mechanical shutter (Vincent Associates) (Fig. 2). Stimulus intensities were comparable to light intensities of $<1 \times 10^{-6}$ to 5×10^{-2} μW cm^{-2} present at daytime depths frequented by *S. similis* in the Santa Barbara Basin (Clarke, 1966).

Photomultiplier recordings

For these long-term experiments, the seawater in the acrylic chamber containing the restrained animal was exchanged at a rate of approximately 50 ml min^{-1}. The apparatus for light stimulation was as described above. Bioluminescence was detected by an EMI 9781B photomultiplier operating at −550 V and fitted with an electromechanical shutter (Fig. 2). The photomultiplier was located 10 cm beneath the animal. The stimulus light and the photomultiplier were isolated by a pair of rotating light choppers (Rofin) producing 5 ms light pulses at 100 Hz, synchronized 180° out of phase with each other and positioned one above and one below the experimental chamber. Consequently, the photomultiplier viewed the specimen in the dark interval between light pulses delivered to the specimen. The test animal perceived the light stimulus as a continuous source, because the chopping rate was greater than the critical flicker fusion frequency of marine crustaceans, which is typically below 60 Hz (Waterman, 1961). The chopped photomultiplier signal was led through a Keithley 427 Current Amplifier, rectified by a Keithley Autoloc 840 Amplifier referenced to the chopping frequency, and displayed on a Grass 79D Polygraph. A photodiode monitored the filtered light stimulus and registered stimulus presentations on the polygraph record.

Specimens in the chamber were acclimated in the dark for at least 20 min following handling under dim red light. They were then subjected to light stimuli ranging from 2×10^{-5} to 4×10^{-4} μW cm^{-2}.

The intensity of bioluminescence was measured from the polygraph record as amount of baseline shift corrected for dark current, and expressed as photomultiplier anode current. The apparatus was not calibrated for luminescent output in irradiance units.

Image intensification

Bioluminescence from restrained animals was viewed from below with an image intensifier (EMI Type 9912, four-stage, maximum radiant power gain 10^6 at 440 nm), fitted with a 75 mm f/1.9 objective lens, by means of a first-surface mirror positioned beneath the chamber at

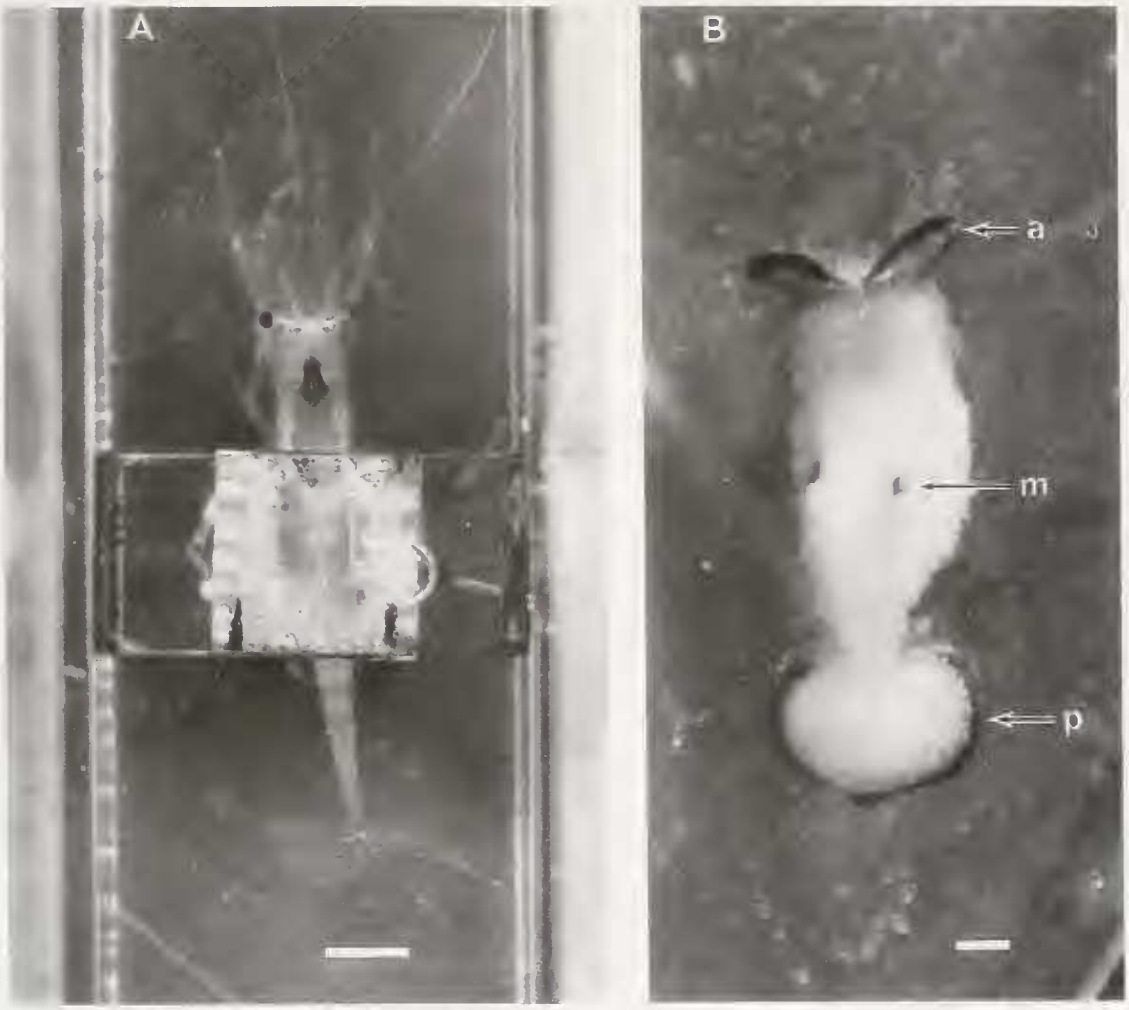

Figure 1. Views of intact and dissected preparations of *Sergestes similis*. (A) Dorsal view of living intact specimen restrained in testing chamber. Specimen was loosely clamped about the midregion of the body during experimentation. Immediately anterior to the clamp is the hepatopancreas and foregut. The chamber was superfused with chilled (10°C) filtered seawater. Scale bar = 5 mm. (B) Ventral view of isolated hepatopancreas showing locations of luminous tissue. Dark pigmentation characteristic of the luminous tissue (arrows) is associated with the (a) anterolateral pair of organs of Pesta, (m) lateral midgastric pair of organs, and (p) posterior fringe organ. Scale bar = 1 mm.

an angle of 45°. Typical operating voltage was 34 kV. The anode phosphor of the image intensifier was viewed by a Panasonic newvicon video camera with a 25 mm f/0.95 objective lens, and images were recorded on videotape together with a time and video frame reference. The apparatus for stimulus illumination was as described above.

The chamber containing a restrained animal was positioned in the dark in the experimental apparatus. During experiments, the stimulus intensity was either 1×10^{-5} or 2×10^{-4} μW cm^{-2}. At one-minute intervals, the stimulus was briefly extinguished to permit documentation of bioluminescence.

Chemical stimulation

The physiological basis of the slow photic induction of bioluminescence was further investigated with tests of putative invertebrate neurotransmitters. For this study, specimens of *S. similis* were collected during the day from the Santa Barbara Basin and thereafter maintained in darkness and handled under dim red light. Intact live animals were restrained in the test chamber. In some cases, the hepatopancreas tissue with attached light organs was isolated by dissection, pinned in a clear dish layered with Sylgard, and placed in the test chamber. In some specimens, both eyestalks were ablated at their bases with iridectomy scissors prior to chemical testing. Biolumines-

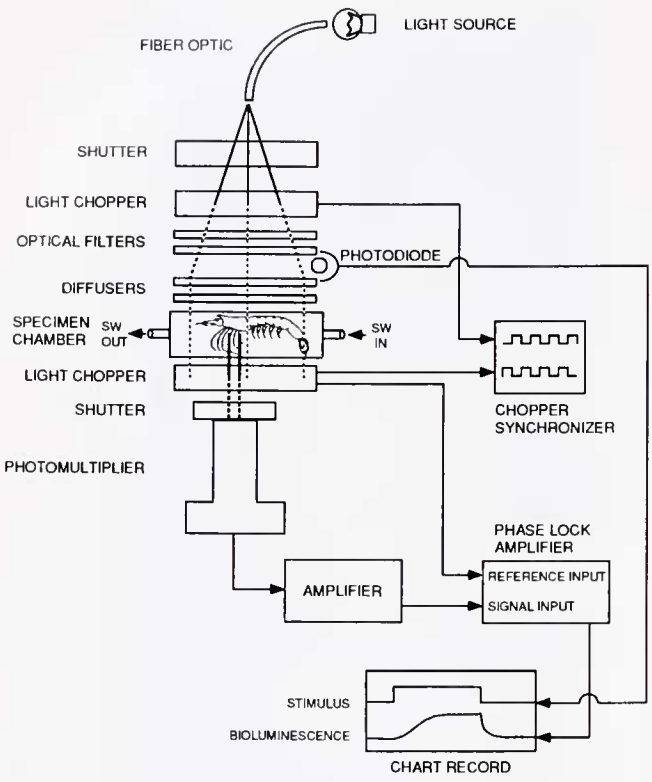

Figure 2. Schematic of experimental apparatus used to measure the intensity of bioluminescence during counterillumination. The specimen in the testing chamber superfused with chilled seawater (SW) was subjected to a diffuse downward-directed illumination of controlled intensity and wavelength, pulsed at approximately 100 Hz by a light chopper (dashed lines above specimen). Downward-directed luminescence (solid lines below specimen) was chopped (dashed lines below specimen) by a second light chopper, synchronized 180° out of phase with the stimulus chopper, and was detected by a photomultiplier. The second light chopper prevented the stimulus illumination from reaching the detector. The bioluminescence signal was amplified, rectified by a lock-in amplifier, and displayed on a chart recorder along with a stimulus record obtained from a photodiode monitoring the stimulus illumination. Not drawn to scale.

cence was detected from below the chamber by an EMI 9701B photomultiplier operating at −750 V and fitted with an electromechanical shutter. The photomultiplier signal was amplified by a Keithley 427 Current Amplifier and displayed on a Grass 79D Polygraph. Levels of light emission were expressed as PMT anode current, without radiometric calibration.

The action of neurotransmitters was assayed with intact specimens or isolated hepatopancreas tissue containing the organs of Pesta. The following solutions were prepared in filtered seawater: 1×10^{-3} *M* acetylcholine (ACh), 1×10^{-3} *M* gamma aminobutyric acid (GABA), 1×10^{-3} *M* L-glutamic acid, 1×10^{-3} *M* norepinephrine, and 5.7×10^{-4} *M* serotonin creatinine phosphate (5-hydroxytryptamine). In addition, the following combinations of serotonin and serotonin-specific chemicals were tested: 5.7×10^{-4} *M* serotonin plus 1.5×10^{-5} *M* cinanserin (Squibb 10,643 cinnamanilide hydrochloride), a serotonin antagonist; 5.7×10^{-4} *M* serotonin plus 1.3×10^{-3} *M* fluoxetine, a serotonin uptake inhibitor; 10^{-4} g/ml p-chloroamphetamine (PCA), a serotonin releasing agent; and 1×10^{-3} *M* 5-methoxytryptamine (5-MT), the precursor to serotonin (see Fuller, 1982). The control consisted of filtered seawater alone. All solutions were prepared in advance and frozen in glass vials in 50 ml aliquots until time of use. For testing, vials were thawed and solutions equilibrated to 10°C prior to filling the experimental chamber. Intact specimens or isolated hepatopancreas tissue were then immersed in the test solution. Permeability of solutions to the site of action was not considered to be a problem with this protocol because it has been successfully used on euphausiids and shrimps treated with serotonin, cinanserin, and other compounds (Herring, 1976; Herring and Locket, 1978).

The kinetics of the luminescent responses were described according to the following terms: latency, the time period from presentation or termination of stimulus to beginning of response; half rise, time from stimulus presentation to half maximum response amplitude; half decay time, time from stimulus termination to half maximum response amplitude. Unless otherwise stated, values are stated as mean ± standard error of the mean.

Parametric statistical tests included the two-sample T test and one-factor analysis of variance, while the Mann-Whitney U test and Kruskal-Wallis test were used for nonparametric comparisons. All statistical tests were performed using Statview software (Abacus Concepts, Inc.).

Results

Photic stimulation

The luminescent response of restrained specimens of *Sergestes similis* to photic stimulation depended upon the degree of recent light exposure. Previously untested animals responded differently from counterilluminating specimens.

The typical counterillumination response to a dim photic stimulus (Fig. 3A) displayed a latency of 2 s and reached half maximum intensity within 13 s (Table I). Generally, steady-state emission was achieved within 25 s. Luminescent intensity remained stable while the stimulus was maintained. Upon termination of the stimulus, luminescence was rapidly extinguished after a latency of 1 s (Table I). The kinetics of the counterillumination responses in the present study were similar to those previously measured (Warner *et al.*, 1979).

These responses were not present in previously untested, dark acclimated specimens of *S. similis*. There was a latency period of several minutes during which no lu-

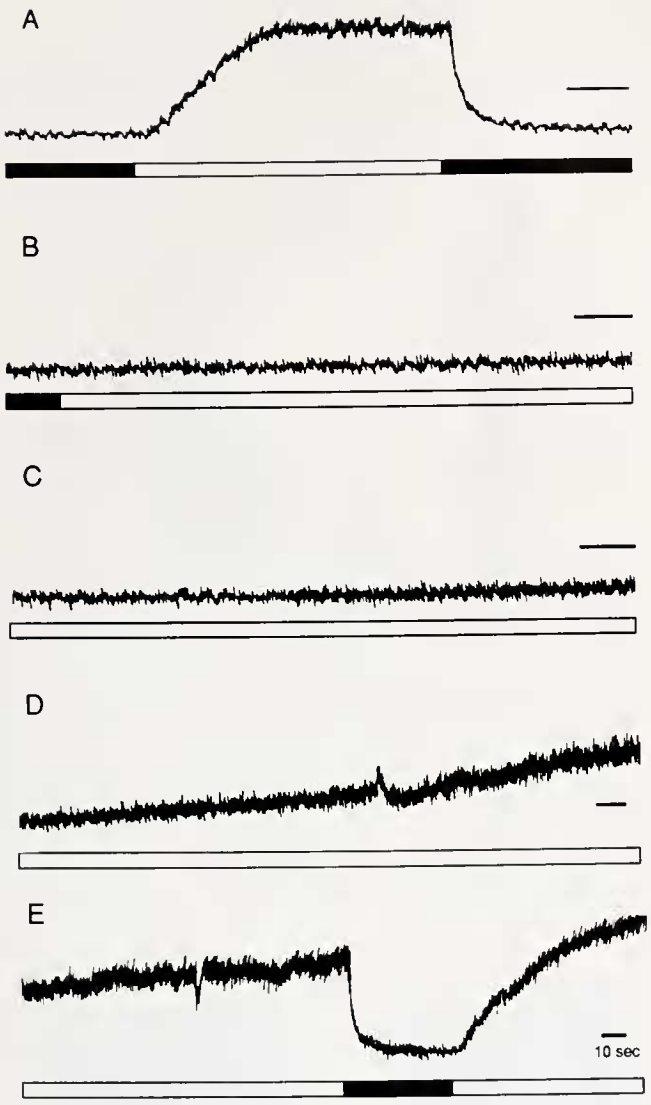

Figure 3. Comparison of counterillumination and slow photic induction of bioluminescence. For each trace of the chart recorder record, the upper trace is the bioluminescence record, with an upward deflection indicated increasing levels of light emission; the lower trace monitors stimulus illumination, with a solid bar indicating no photic stimulus and a clear bar representing stimulus "on." (A) Counterillumination in response to stimulus illumination of 1×10^{-3} μW cm^{-2}. Steady luminescence was produced only when the light stimulus was present, and was rapidly extinguished at the end of stimulation. (B–E) Slow photic induction of bioluminescence in a previously untested, dark-acclimated animal, illuminated by a maintained light stimulus with an intensity of 2×10^{-4} μW cm^{-2} (clear bar). Dim bioluminescence slowly increased in intensity until termination of the stimulus midway through (E) (dark bar), which produced a rapid extinguishing of luminescence. The subsequent photic stimulus (clear bar) resulted in rapid "on" kinetics similar to those of counterillumination in (A).

minescence was produced; subsequently, light emission slowly increased (Fig. 3). Based on photomultiplier measurements, light emission was first detected 3.3 ± 0.7 min (range 2–5 min) after stimulus initiation. Luminescence reached half maximum intensity after 12 min; maximum steady light output occurred after approximately 25 min of illumination (Table I).

Image intensification confirmed that light emission originated from the organs of Pesta (Figs. 1B, 4). Based on observations of 31 previously untested animals, luminescence was induced in the anterior organs 2.4 ± 0.3 min (mean ± standard error) after presentation of the light stimulus, and in the posterior organs 3.6 ± 0.5 min after the beginning of stimulation. Even though there was no statistical significance to the earlier onset of emission by the anterior organs (paired-sample t test, $t = 0.86$, $P > 0.20$), this trend was observed in more than 60% of the specimens tested. In most cases, the anterior light organs were the brightest, and light emission from the midgastric organs was very dim if detected at all.

Once luminescence was induced, an animal was capable of subsequent responses with fast kinetics typical of the counterillumination response. Termination of the initial photic stimulus resulted in a rapid extinguishing of luminescence (Fig. 3E) after a latency of 1 s. The kinetics of the induction "off" response did not significantly differ from those of the counterillumination "off" response (Table I). All subsequent photic stimulation resulted in light emission with rapid response kinetics. An "on" response latency of 2 s and time to maximum intensity of 25 s was similar to those of conventional counterillumination responses. Image intensifier observations under these conditions indicated that once luminescence was induced, the light organs invariably responded synchronously to stimulus "on" and "off."

Photic stimulation was needed not only for induction of the counterillumination response, but also to maintain this state. Preliminary observations indicated that after 1 h of darkness, a previously counterilluminating animal underwent a new induction process similar to those of untested specimens. Subsequent to this, counterillumination was regained.

Eye glow, indicative of the dark-adapted eye state (Ball *et al.*, 1986), was observed prior to testing in 5 of 6 dark-maintained specimens, but was absent after testing. Animals adapted to a light intensity of 1×10^{-2} μW cm^{-2} (an intensity higher than that present in their depth range: Clarke, 1966) did not exhibit eye glow (0 of 4 specimens), suggesting that the eye is light adapted at this level of illumination. The threshold for light adaptation was not determined.

Chemical stimulation

Serotonin was the only neurotransmitter tested that was effective in producing bioluminescence (Fig. 5). Maximum levels of light emission from intact animals immersed in 5.7×10^{-4} M serotonin were significantly dif-

Table I

Kinetics of the luminescent responses of Sergestes similis

Condition	"On" latency (s)*	Half rise time (s)*	"Off" latency (s)†	Half decay time (s)†
Induction	198.0 ± 39.0 (4)	750.0 ± 86.6 (3)	1.3 ± 0.8 (2)	2.5 ± 1.5 (2)
Counterillumination	2.2 ± 0.2 (11)	12.8 ± 1.2 (11)	0.9 ± 0.1 (10)	1.6 ± 0.1 (11)

* Mean values for induction and counterillumination conditions are significantly different (Two-sample T test, $t \geq 9.497$, $P < 0.001$).
† No significant difference between means for test conditions ($t \leq 1.584$, $P > 0.10$).
Values represent means with standard errors of the mean; number of observations given in parentheses.

ferent from seawater controls (Mann-Whitney U test, U = 42, $P < 0.01$). The average temporal response consisted of a latency of 6.0 ± 0.5 min followed by a slow increase to maximum intensity that was reached in 26.7 ± 3.5 min (Fig. 6B). These response kinetics are similar to those for luminescent induction by photic stimulation.

Treatment with the neurotransmitters acetylcholine, GABA, L-glutamic acid, and norepinephrine did not result in levels of bioluminescence significantly different from seawater controls (Mann-Whitney U-test, $P > 0.1$) (Fig. 5).

The specificity of serotonin in stimulating bioluminescence was further investigated (Fig. 5). A solution of serotonin and fluoxetine, a serotonin uptake inhibitor, did not produce significantly higher levels of light emission compared to serotonin alone (Mann-Whitney U test, U = 18, $P > 0.5$), nor did a solution of serotonin and cinanserin, a serotonin antagonist, produce significantly lower levels of light emission (Mann-Whitney U test, U = 18, $P > 0.1$). There was no difference in the response latencies for these conditions from that of serotonin alone (Kruskal-Wallis test, $P > 0.05$). Treatment with PCA, a serotonin releasing agent, and 10^{-3} *M* 5-MT, a serotonin agonist, did not result in significant production of luminescence (Mann-Whitney U-test, $P > 0.2$).

Isolated hepatopancreas tissue containing the light organs produced background levels of luminescence that were significantly higher than for intact animal seawater controls (Fig. 5, 6C) (Mann-Whitney U-test, U = 55, $P < 0.01$). Treatment of the isolated tissue with serotonin did not significantly alter the control glowing (Mann-Whitney U-test, U = 25, $P > 0.5$).

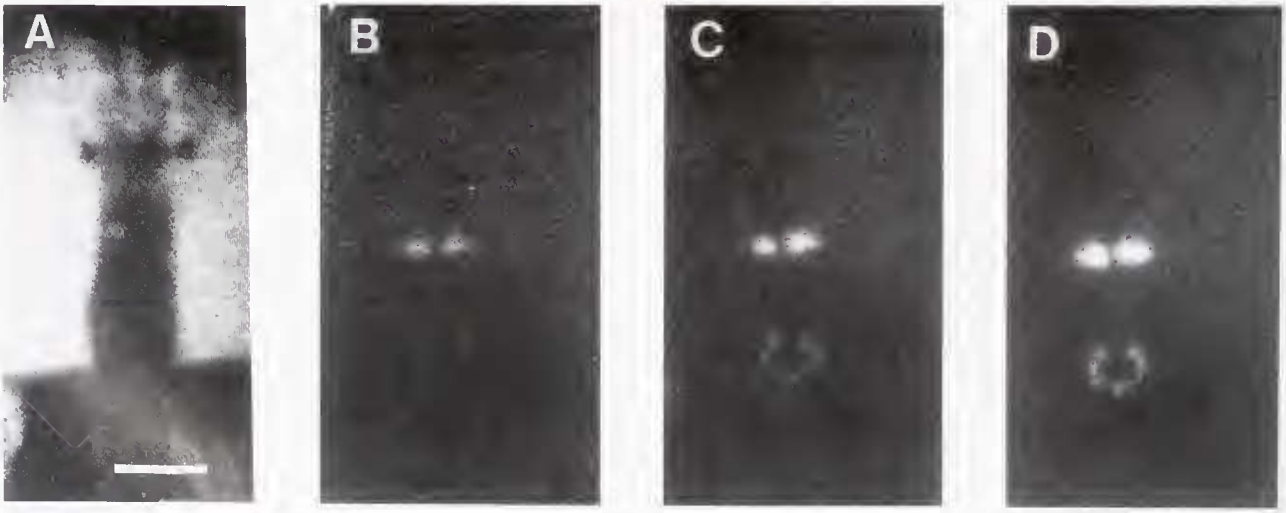

Figure 4. Image intensifier views of slow photic induction of bioluminescence. Photographs of ventral views of an animal were made from single fields of the video record. (A) View of cephalothorax of an intact restrained specimen (clamp at bottom) under dim red light illumination. Anterior end is up. Bioluminescence was observed (B) 2 min, (C) 3 min, and (D) 6.5 min after the beginning of maintained light stimulation, showing emission initially from the anterior organs of Pesta, and then dimmer emission from the posterior light organ. For (B–D) the stimulus light was briefly extinguished for photographic documentation. Scale bar in (A) = 5 mm.

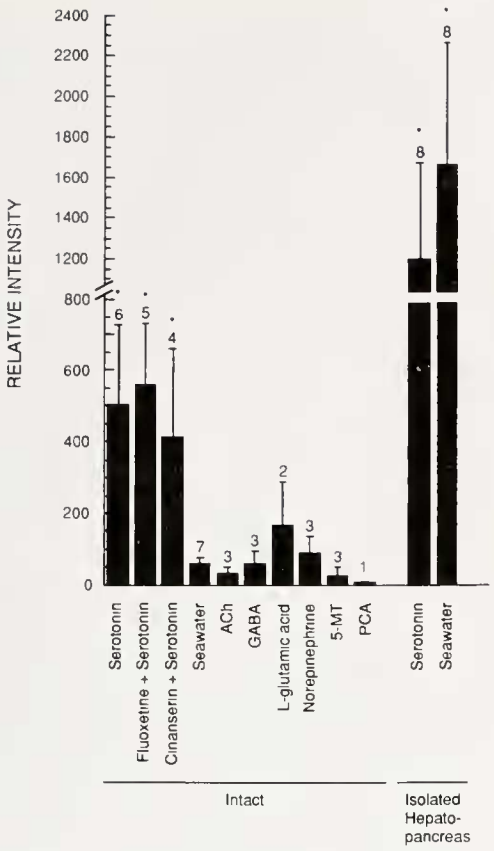

Figure 5. The effect of chemical treatment on bioluminescence. For each experiment, the maximum relative intensity of light emission in the initial 35 min of stimulation was determined. Concentrations of solutions are given in Materials and Methods. Mean intensities with standard errors of the mean are shown for each condition; the number of treatments is displayed above each bar. An (*) indicates that the experimental treatment produced bioluminescence significantly different from seawater control levels in intact animals (Mann-Whitney U test, $P < 0.05$).

Effect of eyestalk manipulation

Squeezing or ablating the eyestalks of previously untested animals immediately evoked luminescence. It was not possible to obtain response latency values as the PMT shutter was closed during the eyestalk manipulation. However, when the shutter was opened 5 s following the procedure, light emission was present.

Serotonin treatment was effective in animals with ablated eyestalks (Fig. 7). In one experiment, eyestalkless animals treated with serotonin produced a higher intensity of light emission than the serotonin-stimulated luminescence of intact specimens (Mann-Whitney U test, U = 40, $P < 0.01$). Squeezing a single eyestalk of intact serotonin-induced luminescing animals immediately increased light emission by more than a factor of 2 (n = 3).

Discussion

The responses to light of previously untested, dark-maintained specimens of *Sergestes similis* clearly differed from the typical counterillumination responses ascribed to this species (Warner *et al.*, 1979). Previously untested animals generated no detectable luminescence for several minutes after initial photic stimulation; subsequently, light emission increased to a maximum and steady level approximately 25 min later. However, once induced, subsequent luminescent responses displayed the rapid kinetics typical of the counterillumination response. The different

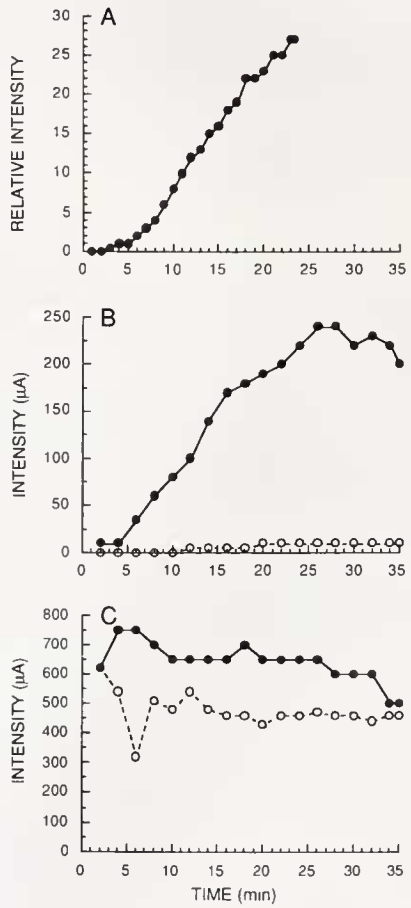

Figure 6. The comparison of slow induction of bioluminescence by photic and serotonin stimulation. The intensity of emission is shown as a function of time of stimulation. (A) The response of an uninduced dark-adapted specimen to initial photic stimulation with a light intensity of 4×10^{-4} μW cm^{-2}. Bioluminescence is expressed in relative units. (B–C) Luminescent responses (expressed as PMT anode current in μA) to treatment with 5.7×10^{-4} *M* serotonin and seawater controls. (B) Responses of intact animals. Serotonin was effective in producing a slow rise in light emission (solid circles), while seawater was ineffective (open circles). (C) Tests with isolated hepatopancreas tissue containing the luminescent organs of Pesta. Serotonin treatment (solid circles) did not increase luminescence above initial high background levels. Seawater control levels (open circles) were higher than controls for intact animals (open circles in B).

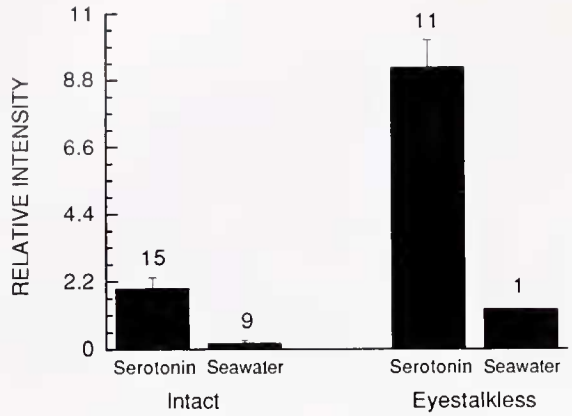

Figure 7. The effect of bilateral eyestalk ablation on bioluminescence. The mean intensity of maximum light emission produced in the first 35 min of stimulation is shown along with standard errors of the mean. All serotonin treatments were significantly different from seawater controls (Mann-Whitney U test, $P < 0.01$). The serotonin response of eyestalkless animals was significantly different from that of intact specimens (Mann-Whitney U test, $P < 0.01$).

kinetics of the induction and counterillumination responses suggest dual control mechanisms regulating light emission.

One mechanism for control of bioluminescence in *S. similis* may involve a neuronal pathway. Evidence for neural control includes: (1) the immediate production of luminescence upon eyestalk ablation of previously untested animals, and (2) the immediate increase in light emission following pinching of an eyestalk of an actively luminescing specimen.

The kinetics of the counterillumination response in *S. similis* are consonant with those of neurally controlled systems. Direct electrical stimulation of the spinal cord of myctophid fishes results in luminescent response latencies of 15 s or less (Anctil, 1972; Barnes and Case, 1974). Intact counterilluminating myctophids exhibit average response latencies of 1 to 18.5 s (Case *et al.*, 1977), with a half rise time of 12 s and a half decay time of 1–2 s (Young *et al.*, 1979). Even though at present there is no morphological evidence for innervation of the organs of Pesta of *S. similis* (Herring, 1981), the kinetics of the counterillumination response of *S. similis* (half rise time of 12.8 s, half decay time of 1.6 s) are similar to those of the neurally controlled myctophid control system.

The long latency and slow increase in emission intensity during the induction process suggest a different control mechanism active during this period. Several features of the induction process support the involvement of a blood-born or neurosecretory pathway: (1) Photic induction of bioluminescence occurred at a similar rate to chromatophore pigment dispersion in crustaceans, where an increase in illumination causes release of erythrophore pigment dispersing hormone (reviewed by Rao, 1985). (2) Bioluminescence is stimulated by serotonin, which is a known crustacean hormone releasing factor (reviewed by Rao, 1985; Fingerman, 1987). (3) The loss of the counterillumination state in *S. similis* after dark re-adaptation may be due to the clearing of a blood-born substance, similar to the return to the dark-adapted state of the crustacean eye via gradual clearing of light-adapting hormone from the hemolymph (Brown *et al.*, 1952). Initial observations confirmed that eye glow in untested specimens of *S. similis*, which indicated a dark-adapted eye state (Ball *et al.*, 1986), was absent after testing, indicating a change to the light-adapted eye state. (4) The induction process did not appear to involve the light-producing ability of the photogenic cells, because the light organs of uninduced specimens produced immediate luminescence upon squeezing or ablating the eyestalks.

A bioluminescence induction process has not been described for other counterilluminating midwater animals. Some species of shallow-living leiognathid fishes of the Indo-Pacific exhibit an initial slow rise in light emission, proportional to the previous period of dark adaptation, although this is due to chromatophore modulation of light organ transparency rather than physiological regulation of the production of luminescence (McFall-Ngai and Morin, 1991; McFall-Ngai, pers. comm.). Perhaps a more analogous phenomenon is arousal in the firefly *Photuris*, which, if stimulated during daytime, requires 15 to 30 s before flashes can be generated. During this period, the light organ glows with increasing intensity and, finally, flashing capability is established just after a rapid quenching of the glow (Case and Buck, 1963).

The adaptive significance of an uninduced state and the slow induction of bioluminescence is obscure. *S. similis* does perform diurnal vertical migrations (*e.g.*, Clarke, 1966; Pearcy and Forss, 1969; Omori and Gluck, 1979) during which it apparently follows a particular isolume (Clarke, 1966). Continuous exposure to dim downwelling illumination would serve to maintain animals in the active counterilluminating condition. On moonless nights, when levels of downwelling illumination would be undetectable and counterillumination unnecessary, animals would revert to the uninduced condition. This might prevent inadvertent luminescent responses to the luminescent displays of other animals and thereby reduce the chance of being detected by predators. Although *S. similis* can respond to light pulses as short as 2 s in duration (Warner *et al.*, 1979), it is not known if it responds to shorter duration stimuli typical of luminescent flashes.

The role of light in the induction of counterillumination in *S. similis* differs from light pulses that produce bursts of luminescence in some organisms. For the shrimp *Thalassocaris* (Herring and Barnes, 1976), copepod *Metridia longa* (Lapota *et al.*, 1986), ostracods (Tsuji *et al.*, 1970;

Morin, 1986), and pyrosomes (Bowlby *et al.*, 1990) a photic stimulus acts as a trigger to release luminescent behavior. In contrast, the long time course of luminescent induction in *S. similis* suggests a longer-term change in physiological state occurring during the induction process.

Salient features of the *S. similis* luminescent system are similar to those of euphausiids. In euphausiids, light emission is stimulated by bright light or strobe illumination after a latency of several minutes. Serotonin is the only neurotransmitter that stimulates light emission in euphausiids, with a latency of 5 to 15 min (reviewed by Herring and Locket, 1978). This response occurs only in intact animals; isolated photophores treated with serotonin do not luminesce (Herring and Locket, 1978). Although the euphausiid control system has not been fully elucidated, it is believed to involve control of blood flow through the photophores by innervated sphincters (Harvey, 1977; Herring and Locket, 1978).

Serotonin is present in the tissues of many marine invertebrates (reviewed by Walker, 1984), and has been detected in the eyestalks, cerebral ganglia (brain), ventral nerve cord, and hemolymph of crustacea (*e.g.*, Fingerman *et al.*, 1974; Elofsson *et al.*, 1982; Laxmyr, 1984). It is well known to act on the crustacean neuromuscular junction by increasing neurotransmitter release (reviewed by Kravitz *et al.*, 1985). Serotonin also acts on neurosecretory cell terminals in the sinus gland of the crustacean eyestalk. It mediates the release of a putative neurodepressing hormone, a putative molt-inhibiting hormone, the hyperglycemic hormone, and a red chromatophore pigment dispersing hormone from neurosecretory cells in the eyestalk (reviewed by Rao, 1985; Fingerman, 1987). The pigment dispersing hormone is effective only in intact animals; direct treatment of serotonin on erythrophores in isolated legs or carapace has no effect (Nagabhushanam *et al.*, 1987; reviewed by Fingerman, 1987). This hormone also acts to cause migration of the retinal distal pigment to the light-adapted state (Kleinholz, 1975).

There are no marine luminescent systems in which direct hormonal control of light emission has been demonstrated. Direct innervation of squid and euphausiid light organs occurs even when the photophores receive a rich blood supply through an extensive capillary network (Arnold and Young, 1974; Herring and Locket, 1978). Control of leiognathid bioluminescence through muscular shutters may be fine-tuned through the action of chromatophores with slow response times (McFall-Ngai and Morin, 1991), which are presumably under neural control.

The present data suggest at least two sites involved in the control of bioluminescence in *S. similis*. The eyestalk contains the photoreceptors that detect downward-directed illumination, and associated efferent neural or neurosecretory cells. The responses of eyestalkless animals to serotonin suggest an additional control site, possibly located in the central ganglia. Furthermore, spontaneous light emission from isolated light organs suggests inhibitory control of light emission. The close coupling of vision and bioluminescence in *S. similis* may be achieved via a hormonal component simultaneously active in the visual and luminescent systems.

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